A SERO-SURVEY (IMMUNOFLUORESCENT ANTIBODY TECHNIQUE) OF CONGO, RIFT VALLEY FEVER, EBOLA, (ZAIRE AND SUDAN), LASSA AND MARBURG VIRUSES IN THE RIVER BENUE BASIN, IN NIGERIA

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BY

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ABSTRACT

A retrospective study was done in the River Benue Basin of Nigeria, in which a serological survey of randomly stratified serum samples from the locality was done, using the indirect immunofluorescent technique. Four hundred seventy serum samples were retrospectively examined for polyvalent (Congo, Rift Valley Fever virus, Ebola Sudan, Ebola Zaire, Lassa and Marburg) CRE_2LM viral immunofluorescent antibodies. One hundred ninety three were positive to the polyvalents, i.e., 41 percent positive. Monovalent antibodies were then tested for, from the polyvalent positives.

In the study for Congo virus antibody 116 of 193 (60%) of CRE_2LM positives were tested, 10 were positive of the 49 males tested (20%). Females, 22 were positive of the 67 tested (33%). Projected value of 14% of the total samples will be positive.

In the Rift Valley Fever virus study 158 of 193 (81%) of CRE_2LM positives were tested, 11 of 63 (17%) males were positive, and 14 of 95 females were positive (15%). A projected value of 5% of the total sample population is positive.

Ebola Sudan and Ebola Zaire data were merged, there were 113 of 193 CRE_2LM positives tested (58%). Fourteen of 113 tested for Ebola (Zaire and Sudan) were positive (12%)/ Ebola Zaire monotypic, 7/113 (6%) and Ebola Sudan monotypic 37 (33%) were positive. A projected value of 19 percent of the total sample population is positive to Ebola virus antibody.

In the Lassa virus antibody study, 190 of 193 (98%) of CRE_2LM positives were tested, 15 of 78 (19%) males and 31 of 112, (28%) females were positive. A projected value of 9.8% of the total sample population is positive.

In the Marburg virus antibody study, 166 of 193 (86%) of CRE_2LM positives were tested, 2 of 66, (3%) males and 5 of 100, (5%) females were positive for Marburg virus antibody. A projected value of 1.4 percent of the total sample population has Marburg antibody.

It is evidenced from the above, that Congo, Rift Valley Fever, Ebola (Sudan), Ebola (Zaire), Lassa and Marburg Viruses were present in the River Benue Basin. The author considers these results to suggest that, under favorable conditions, the above mentioned viruses are a threat to human health in Nigeria. The sex differences seen are not statistically significant $p>0.05$, sampling variation is a likely explanation of the differences. There were also no geographic, occupational, rural/urban differences, as all these factors were the same to the area studied.

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TABLE OF CONTENTS

CHAPTER 4

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

A serological survey, (using the immunofluorescent antibody technique), of exotic viruses in Nigeria (Congo, Rift Valley Fever, Ebola (Zaire) Ebola (Sudan), Lassa and Marburg Viruses) was embarked upon to identify areas of prevalent viral disease in the River Benue Basin. An retrospective study was done, the main objective was to show that these viruses are present in the area studied.

The author developed interest in doing work in this field because, being a physician, he had on several occasions managed patients with fever of unknown origin (F.U.O.) in Lagos, Nigeria, without really getting to the root of the problem. This was a way to find out the etiology of F.U.O., especially of viral origin.

The diseases caused by these viruses, because of the difficulty in virus identification and disease diagnosis have been shelved under the umbrella disease "malaria". Undiagnosed febrile illnesses that have failed to respond to antimalarial management have been suspected to have other etiology, especially viral. However, due to inadequate laboratory diagnostic techniques for viral diseases, it has been difficult to establish the exact diagnosis and extent of exotic viral disease.

The exotic nature of these viral diseases made it imperative to try to show that these diseases are in fact present in the area studied and that they do in fact cause morbidity and mortality. A

World Health Organization (WHO) study embarked upon in Northern Nigeria for the prevalence of yaws and malaria, also included the study of these exotic viruses. But for the foresight of the W.H.O. team, one of their team, a physician, had suggested that the sera collected from their study be stored for further work in the future. Some years later the polyvalent antigen technique was discovered and it was then used for this present study. (Discussion with *Dr. Downs) Our study was therefore done on sera previously collected.

Lassa fever had not been reported when the initial study was done. This was of particular significance in that it showed that Lassa fever was present, but not in the full blown clinical form. This presentation will give a brief geography of Nigeria, which contains the River Benue Basin.

Nigeria lies within the tropics between latitudes 4° and 14° north of the Equator and longitudes 3° and 14° east of the Greenwich meridian. It is bounded on the west by the Republic of Benin, on the north by the Niger Republic, on the east by the Republic of the Cameroons and washed on the south by the Atlantic Ocean.

The country, which has an area of 913,072.64 square kilometers, is well watered by the Rivers Niger and Benue and their tributaries. Its climate varies from tropical at the coast to sub-tropical further inland. There are two well marked seasons -- the dry season lasting from November to March and the rainy season from April to October. Temperatures at the coast seldom rise above 32°C, but humidity can be as high as 95%.

*Wilbur G. Downs, Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

The climate is drier further north where extremes of temperatures are common, sometimes ranging from 36°C to 12°C.

Rainfall is usually heavy in the south, averaging about 177.8 cm. a year at the western end of the coast and increasing to about 431.8 cm. along the eastern section of the coast. The rainfall decreases fairly sharply inland and is around 127 cm. over most of central Nigeria, falling to 50.8 cm. in the extreme north.

Nigeria's vegetation can be divided into two main and easily recognizable sections: the high forest zone which covers one-sixth of the country and the savannah.

Nigeria's population of about 80 million is multi-ethnic. Among the principal ethnic groups are the Hausa, Fulani, Ibo, Yoruba, Edo, Efik, Jaw, Tiv and Kanuri.

The River Benue basin is limited to the west by the River Niger, and to the south by the border between the eastern and northern regions and includes roughly the division located on both sides of the Benue River.

The political entity known as Nigeria came into formal existence in 1914 with the amalgamation of the northern and southern British protectorates. Before this date, the part of the Sudanic Belt which the country now occupies had a history that was essentially the story of the migration and fusion of people, the rise and fall of empires, the slave trade and its replacement by the legitimate trade in tropical produce, the establishment and expansion of British influence in both the northern and southern parts of the country and finally, the imposition of British rule which culminated in the birth of a nation.

With the amalgamation of the separate administration of northern and southern protectorates on January 1, 1914, the colony and protectorate of Nigeria came under a unitary administration presided over by a Governor-General. For administrative convenience, however, the country was divided into four, namely the Colony of Lagos and the Northern, Eastern, and Western Provinces. Today, however, there are nineteen states, ruled over by a presidential system of government.

General Problems Addressed by This Study

Of the one thousand eight (1,008) serum samples tested, six hundred ninety four (694) had demographic information on them and also appeared on the master list for the study, hence three hundred fourteen (314) tested sera data were excluded from the study.

CHAPTER 2

REVIEW OF STUDIES RELEVANT TO THE PROBLEM

Lassa Virus

"But Africa has had a nasty habit recently of turning up new and dangerous virus diseases such as Lassa, Marburg and Ebola..." (1).

Lassa virus has received more international attention since it is commonly associated with man-to-man transmission, particularly within hospitals.

It is a member of the family arenaviridae. Members of the arenavirus taxon take their name from their unique appearance under the electron microscope: several inclusion-like dense particles give the viruses a sand-sprinkled (arenosus), appearance. Arenaviruses have rodents or bats as their natural hosts and reservoirs, in which they produce either immunity or a persistent tolerant infection; The persistently-infected rodent suffers no ill effects, and develops no immune response, though during its life-time the animal excretes virus, particularly in the urine (48). Lassa virus is an RNA-containing virus and has enveloped coiled nucleocapsids. The virion has a diameter of 80-130 nm.

History

Lassa fever was first observed in 1969, in an American missionary nurse stationed at Lassa in Northeastern Nigeria. Following her admission to a hospital in Jos, Nigeria, two contact cases developed in

nurses at that hospital (2). Because of the circumstances surrounding this outbreak and the fact that two of the three persons affected died, the disease acquired from the outset a reputation for severity that subsequent events amply justified. In addition to the initial episode, four more outbreaks occurred between 1970 and 1974 in Nigeria, Liberia, and Sierra Leone (3,4,5). Furthermore, laboratory accidents have occurred in the United States (46,47). Serological surveys suggest that the infection also occurs in Guinea and the Central African Empire (1). Among hospital patients the case fatality rate is about 36%, but in the general community it may be as low as 3%-5% (9).

Descriptive Epidemiology: Geographic Distribution

The disease has been observed in several localities in Nigeria since 1969 (Lassa, Jos, Onitsha, Zaria), in Liberia in 1972, and in Sierra Leone since 1970-1972.

Epidemic Types: Distribution by Sex, Age and Season

Since the initial outbreak at Lassa in 1969, during which one of the patients was transferred to New York City, there have been other outbreaks near Jos in Northern Nigeria in 1970 (32 suspected cases with 10 deaths), in Zorzor, Liberia, in 1972 (11 cases with 4 deaths), and in the Eastern Province of Sierra Leone with 63 suspected cases admitted to two hospitals between 1970 and 1972. In Jos and Zorzor, outbreaks apparently resulted from person-to-person nosocomial spread from the index case to

hospital workers or other patients. In Sierra Leone, the great majority of cases were acquired outside the hospital, although hospital workers were at high risk. This clearly delineates two types of outbreaks. The first type, hospital-associated develops as a result of exposure and spread from a hospitalized index case to other patients, visitors, and medical staff. The index case is usually acquired in the nearby community; between 10 and 20 days after admission to the hospital, a cluster of secondary cases develops (6,7,8). This type of outbreak has been the rule, with one exception.

The second type of outbreak, of which there is at present only one example (Sierra Leone, 1970-1972), occurs in the community at large. Patients acquire their infection at home or other community surroundings, rather than by exposure or contact in the hospital with another patient. However, there is also the possibility of nosocomial transmission in this type of outbreak, particularly to the hospital staff (9).

Tertiary cases have been recorded; with a few exceptions - notably by transmission to medical staff - they have been milder. No evidence of further propagation has been reported (6).

The mortality in hospitalized cases has been between 20 and 66% in different outbreaks, with an average of 36%. The mortality following all types of infection as contrasted with severe illness requiring hospitalization, however, appears to be much lower. In the Sierra Leone focus, many persons with antibodies were found who either had not been ill or possibly remembered a mild disease; the overall mortality from Lassa virus

infection in that area may have been only 3-5% (9). There is a seasonal distribution of hospital-associated outbreaks, which have occurred from January to April during the dry season; the Sierra Leone cases occurred through the year, with somewhat higher incidence in the wet season.

Considering age and sex distribution, the Sierra Leone outbreak with its pattern of transmission in the villages showed that there was no significant predilection in morbidity or in case-fatality rates (9). The hospital-associated outbreaks revealed that the distribution by sex and age was determined largely by the characteristics of the exposed population; physicians and nurses have been particularly affected. An antibody survey on more than 800 staff members of Liberian hospitals showed that midwifery students and clinical laboratory workers were the groups at highest risk (10).

Mechanism and Route of Transmission

Spread of the disease in hospital set up is from person to person by either the contact or the airborne route, including direct contact, droplet spread, or sharing of drink, food, clinical instruments, objects, and utensils. The same mode of transmission may be at work in contact infections acquired in the home.

In the community-centered outbreak at Sierra Leone, there was definite clustering of illness and sero positivity without illness in certain households; explained by multiple instances of human infection from the same natural source or by person-to-person transmission following a house index case acquired from the reservoir (9).

The mode of transmission to man from the natural source or reservoir - a rodent - Mastomys Natalensis, is still unknown. It may include direct contact with the rodent, its urine and oral secretions, eating of uncooked rodent flesh, or contact with food and drink contaminated by the rodent, it could also be airborne. There are a number of cases which have been acquired through accidental autoinoculation with needles while starting intravenous fluids. At least one laboratory acquired infection has occurred. In Sierra Leone, 6% of the population surveyed had complement - fixing antibody against Lassa virus, while only 0.2% had recognized disease, suggesting mild disease or inapparent infection. In Liberia 10% of hospital personnel had antibodies. Positive sera were also obtained from individuals in Cameroons and Benin Republic.

Symptoms

The incubation period is between 6 and 14 days. The onset of illness is insidious, with chills, malaise, headache, myalgia, and nausea followed by fever, conjunctival infection and suffusion, and exanthem and edema of the face, neck, and upper thorax. Petechiae and lymphadenopathy are common. After a few days the features get appreciably worse, with the development of hypotension, oliguria and haemorrhages, which may lead to death. An exanthem is often seen early, but a constant finding with Lassa alone is a pronounced pharyngitis. Recent unpublished findings by J. B. McCormick, working in an endemic area of Sierra Leone, have shown that roughly half of the febrile patients attending hospitals have Lassa infection, though few develop serious disease.

Laboratory Diagnosis

The distribution of the virus is geographically limited. It grows readily in cell culture, and the most rapid means of serological diagnosis is the fluorescent antibody technique. Complement fixing antibodies appear late, and this test is much less specific (48).

In an endemic area, an illness characterized by unremitting fever with temperatures of 100°F or higher, persisting for 5-7 days or more, unresponding to antibiotics and antimalarial drugs, accompanied by pharyngitis, malaise, toxic appearance, leukopenia, and later, by facial edema, must give rise to a strong suspicion of Lassa fever.

The virus is isolated from the blood usually between the 3rd and 14th days of illness, less frequently from throat washings, pleural effusions, and urine. Because of the recognized danger inherent in handling the virus, work with it, including attempts to isolate it is restricted to laboratories with special high-containment facilities that will on request undertake to isolate and identify Lassa virus from clinical specimens are: Special Pathogens Branch, Virology Division, CDC, Atlanta, Georgia, U.S.A., and Centre for Applied Microbiology and Research, Porton Down, Wiltshire, England.

Detection of antibodies between early and late samples of sera, by fluorescent antibody or complement fixation test, is a useful diagnostic procedure but not helpful for an early diagnosis, since antibodies are not usually detected before the 19th day after onset by fluorescent antibody or before the 18th or 20th day by complement fixation.

Currently, the fastest way of establishing a diagnosis is by inoculation of the clinical specimen, usually acute phase serum, into several Vero-cell cultures followed by daily examination of a culture by the fluorescent antibody test. Under favorable circumstances a positive diagnosis can be reached in 2 days (34). Detection of antigen in conjunctival scrapings is even a more rapid diagnostic procedure (49).

Rift Valley Fever Virus (RVFV)

Rift Valley Fever virus (RVFV) is a member of the Phlebovirus genus of the family Bunyaviridae (Bishop, et al., 1980) (50) and has similar structural properties to other members of the family: three RNA segments, designated L,M and S (large, medium and small) with mol. wt. of $2.7x10^6$, $1.6x10^6$ and $0.6x10^6$ respectively, two major glycoproteins, G1 and G2, with mol. wt. of $65x10^3$ and $56x10^3$ respectively and a non-glycosylated nucleocapsid protein with a mol. wt. of $25x10^3$ (Rice, et al., 1980) (51).

Information on replication of the virus is limited and non structural proteins are unknown (Bishop, et al., 1980). However, eosinophilic intranuclear inclusions described previously in RVFV-infected cells were shown to fluoresce specifically in an indirect technique with immune sheep serum (Swanepoel and Blackburn, 1977) (52).

This virus causes a severe disease characterized by hepatitis and high mortality in lambs and calves and abortions in ewes and cows. (Daubney, et al., 1931). The disease was known to produce a mild-to-

severe influenza-like illness in man sometimes with serious ocular sequelae (Freed, 1961). In recent years, RVFV disease has assumed public health significance with severe outbreaks of the disease in South Africa, Egypt, and Rhodesia, resulting in human fatalities on a significant scale (Alexander, 1951; Weiss, 1961; Barnard and Botha, 1977; Abdel-Wahab, et al., 1978; Meegan, 1979; Laughlin, et al., 1979; Swanepoel, et al., 1977) (14,16,40).

Human deaths following natural RVFV infection were first recognized in South Africa in 1975, when seven patients died of acute hemorrhagic fever associated with necrosis of the liver (McIntosh, 1975, unpublished information; Van Velden, et al., 1977). In 1977 there was a severe outbreak of RVFV disease in Egypt involving man and animals. The disease was first recognized in man, and, was characterized by a dengue-like illness, with severe cases complicated by either hemorrhage and jaundice, or meningoencephalitis, or retinitis. In a more recent RVFV outbreak in Rhodesia, Swanepoel, et al., (1979) reported five fatal cases of RVFV infection in man, with symptoms identical to those of the Egyptian outbreak.

In Nigeria, RVFV was first isolated from a merino sheep imported from South Africa (Ferguson, 1959). Three more strains of the virus were isolated from Culicoides and Culex antennatus mosquitoes (Lee, 1970).

In a survey for RVFV neutralizing antibodies in the Nigerian livestock population, Fagbami, et al., (1973) demonstrated antibodies in the sera of the following species; camel, goat, sheep, and cattle (but not in pig and horse). The highest prevalence of RVFV antibodies was in the camel (12.0%) followed by goat (9.0%), sheep (3.2%) and cattle (1.6%). In an experimental infection study Tomori (1979a) showed that the indigenous sheep of Nigeria, the West African dwarf sheep, was highly susceptible to infection with RVFV. Despite these observations, there has been no report of any clinical RVFV disease in any indigenous stock in Nigeria. No information is available regarding RVFV disease in man in Nigeria, neither has there been any reports of clinical infection nor has any survey for RVFV antibodies in man been reported. Attempts were made to isolate RVFV from human blood or serum samples collected from different parts of Nigeria, while sera were tested for RVFV antibodies.

Marburg and Ebola Diseases

Marburg and Ebola viruses are morphologically similar, immunologically distinct rod-shaped agents of African origin.

History - Ebola Hemorrhagic Fever in Sudan, 1976

A large outbreak of hemorrhagic fever (subsequently named Ebola

Hemorrhagic Fever), occurred in Southern Sudan between June and November, 1976. There was a total of 284 cases; 67 in the source town of Nzara, 213 in Maridi, 3 in Tembura, and 1 in Juba. The outbreak in Nzara appears to have originated in the workers of a cotton factory. The disease in Maridi was amplified by transmission in a large, active hospital. Transmission of the disease required close contact with an acute case and was usually associated with the act of nursing a patient. The incubation period was between 7 and 14 days. Although the link was not well established, it appears that Nzara could have been the source of infection for a similar outbreak in the Bumba zone of Zaire.

In this outbreak Ebola Hemorrhagic Fever was a unique clinical disease with a high mortality rate (53% overall) and a prolonged recovery period in those who survived. Beginning with an influenza-like syndrome, including fever, headache, and joint and muscle pains, the disease soon caused diarrhea (81%), vomiting (59%), chest pain (83%), pain and dryness of the throat (63%), and rash (52%). Hemorrhagic manifestations were common (71%), being present in half of the recovered cases and in almost all the fatal cases (43).

Two strains of Ebola virus were isolated from acute phase sera collected from acutely ill patients in Maridi Hospital during the investigation in November, 1976. Antibodies to Ebola virus were detected by immunofluorescence in 42 of 48 patients in Maridi who had been diagnosed clinically, but in only 6 of 31 patients in Nzara.

Of Maridi case contacts, in hospital and in the local community, 19% had antibodies. Very few of them gave any history of illness, indicating

that Ebola virus can cause mild or even subclinical infections. Of the cloth room workers in the Nzara cotton factory, 37% appeared to have been infected, suggesting that the factory may have been the prime source of infection.

Ebola Hemorrhagic Fever in Zaire, 1976

Between 1 September and 24 October 1976, 318 cases of acute viral Hemorrhagic Fever occurred in Northern Zaire. The outbreak was centered in the Bumba Zone of the Equator region and most of the cases were recorded within a radius of 70 Km. of Yambuku. There were 280 deaths, and only 38 serologically confirmed survivors.

The index case in this outbreak had onset of symptoms on 1 September 1976, five days after receiving an injection of chloroquine for presumptive malaria at the outpatient clinic at Yambuku Mission Hospital (YMH). He had a clinical remission of his malaria symptoms. Within one week several other persons who had received injections at YMH also suffered from Ebola Hemorrhagic Fever, and almost all subsequent cases had either received injections at the hospital or had had close contact with another case. Most of these occurred during the first four weeks of the epidemic, after which time the hospital closed, 11 of the 17 staff members having died of the disease. All ages and both sexes were affected, but women 15-29 years of age had the highest incidence of disease, a phenomenon strongly related to attendance at prenatal and outpatient clinics at the hospital where they received injections. The overall secondary attack rate was about 5%. Although it ranged to 20% among close relative such as spouses, parents or children, and brother or sister.

Intensive search failed to detect definitive evidence of a link between an epidemic of the disease in Zaire and Sudan.

Both the incubation period, and the duration of the disease averaged about one week. After 3-4 days of non-specific symptoms and signs, patients typically experienced progressively severe sore throat, developed a maculopapular rash, had intractable abdominal pain, and began to bleed from multiple sites, principally the gastrointestinal tract.

This syndrome was caused by a virus morphologically similar to Marburg virus, but immunologically distinct. It was named Ebola virus.

These viruses have the briefest of histories. Information to be presented is derived largely from four rather dramatic epidemics. Marburg virus was first isolated during an epidemic in laboratory workers processing kidney cells from African monkeys in 1967. Cases occurred in Marburg, Frankfort am Main, Germany, and Belgrade, Yugoslavia. A second focal outbreak took place in South Africa in 1975.

Ebola virus was discovered nearly at the same time during 1976 in Zaire and Sudan, in association with epidemics comprising more than 500 cases.

Morphology and Morphogenesis

Marburg and Ebola viruses are indistinguishable large rods, about 80-90 nm in diameter but varying in length from 600 nm to several micrometers. Brush-like spikes protrude from an outer virionic membrane. Branching forms are commonly seen, as are twisted rods and bulbous protrusions at ends of particles. These viruses contain RNA, have essential

lipids on their surface membranes, and contain an internal helical core that is presumed to be the nucleocapsid.

Morphogenesis occurs in the cytoplasm of infected cells, inclusions composed of a matrix containing nucleocapsids are formed, and virus maturation and release occurs by budding through the host-cell plasma membrane. Marburg is now classified in the proposed family filoviridae.

Physical Properties

Marburg and Ebola virus are moderately thermolabile. The virions have a bouyant density of about 1.14 gm in potassium tartrate gradient.

Chemical Properties

These viruses are inactivated by brief exposure to a variety of chemicals. For example, phenol, peracetic acid, sodium hypochlorite and ether. Ebola contains a single, biologically negative strand of RNA with a molecular weight of 4.6×10^6.

Clinical Features

Marburg and Ebola infections are marked by the appearance of headache, progressive fever, sore throat, myalgia and diarrhea. Conjunctivitis is sometimes present, as is a papular exanthem of the palate. By the 4th and 5th day of evolution, there is chemical evidence of hepatitis and most patients develop a centripetal maculopapular rash that rarely lasts more than 3 days. Some patients develop symptoms suggestive of acute pancreatitis. Weight loss, asthenia, and psychological depression are common features of a convalescence requiring several weeks. In

fatal cases, melena, hematemesis, and bleeding from other sites generally begin on the 5th or 6th day of disease, and such paients rarely survive beyond the 9th day. There is ample evidence of disseminated intravascular coagulation.

Diagnosis

In fatal cases, electron microscopy can be expected to reveal virus particles in liver;surviving patients generally develop specific IgM and IgG antibodies about 7-10 days after onset of symptoms. CF antibodies generally appear about 1 week later. To date, no satisfactory method for measuring virus -- neutralizing antibodies has been developed. Antibodies appear sooner, reach higher levels, and persist for much longer when measured by the indirect immunofluorescent technique.

Congo Virus

Crimean Hemorrhagic Fever (CHF) was first recognized in the southern USSR as a clinical entity in 1944. The causative virus was not finally isolated until the mid-1960's. In the meantime, workers in the Congo and Uganda had isolated several virus strains from febrile human beings, to which the name "Congo Virus" was applied. Casals showed in 1969 that the two viruses were indistinguishable.

The situation in Africa is still a challenging unknown. After the initial finding of several human cases in the 1950's, no additional cases have been found. Yet the known range of the virus has been expanded enormously, with numerous isolations from ticks, cattle, goats, and hedgehog, and from Culicoides (biting gnats) in Nigeria and from

ticks in Senegal. The disease in man remains to be described in West Africa.

Crimean-Congo Hemorrhagic Fever virus (CCHF) belongs to the family Bunyaviridae. It is in the Nairovirus genus (Casals and Tignor) (53). CCHF virus is generally a zoonosis circulating unnoticed in nature in an enzootic tick-non human vertebrate-tick cycle. Humans become infected in caves, homes, or other buildings, in areas surrounding their homes, or when wandering (a) some or (b) a great distance away from their usual environment (Hoogtraal, 1979) (22).

Fifteen of 16 virologically proven CCHF cases in Africa survived after courses of illness ranging from mild to severe (Simpson, et al., 1967; Knight, et al., 1968; Robin, 1977). Most evidence that the virus occurs in Senegal, Nigeria, Central African Empire and Ethiopia is based on isolates from ticks from domestic animals, or less often, on isolates from domestic or wild animals. Antibodies to CCHF virus were detected in 24 of 250 febrile human sera from Ibadan, Nigeria (David-West, et al., 1974) (54). Eighteen of the 27 sera were from children 14 years old or younger. Those authors believe that, under favorable circumstances, the virus presents a threat to human health in the Ibadan area. Except for this single study, there have been no surveys of human sera in Africa south of the Sahara. The "sudden" appearance in 1976 of human CCHF in two widely separated areas of Pakistan where the only previous evidence of the virus was two isolates from ticks, is a striking example of the unreliability of currently available CCHF virus data for predicting morbidity rates. Hoogtraal (1979)(22) points out that the very recent histories of Lassa, Marburg and Ebola viruses in Africa are

poignant signals of how much remains to be learned about zoonotic viruses in relation to human disease on the African continent.

Epidemiologic Description

Season, Sex and Age Distribution

Approximately 30 cases of CCHF have been recorded annually in each of the known areas of occurrence in the U.S.S.R. The cases occur between April and September. The sex distribution of CCHF is equal and 80% of the cases occur in the 20 to 60 year age group. The major arthropod vectors for transmission to humans are ticks which belong to the genus Hyalomma.

Reservoir

Cattle and wild hare appear to be reservoirs. Detailed epidemiology has yet to be defined.

A review of epidemiology of tick-borne CCHF in Nigeria (22).

The Rockefeller Foundation supported a program, based at the University of Ibadan, for surveillance and study of arbovirus infection of Nigerian vertebrates from 1964 to 1970 (23,24). CCHF virus infections were demonstrated in ticks, domestic animals, wild mammals, and a stray culicoides midge taken in a light trap in a cattle barn. There were no isolates from humans, and no sero epidemiological surveys for human infections with CCHF virus were attempted during this program (25). However, in 1973-1974, David-West, et al., (1974), detected antibodies in 24 of 250 sera from febrile humans (9/141 male, 15/109 female) at the Ibadan University College Hospital. In the 0 to 14 year age group, 18 sera were

positive (4/84 male, 14/79 female). The authors considered these results to suggest that, under favorable conditions,
to human health in Nigeria.

Nigerian virus strains sent to the Yale and Moscow laboratories (as Congo virus) in the early stages of the Nigerian program proved timely and significant in establishing the common identity of
from Eurasia and Africa.

The 1964-1965 data (28) and the 1964-1968 data (29) on 27 strains from Nigeria ticks provided valuable information. The 24 strains from ticks from cattle were from Amblyomma variegatum, boopnilus decoloratus, hyalomma marginatum rufipes. Hyalomma impeltatum, and Hylamma truncatum. Three additional strains were from B. decoloratus from sheep and from Hyalomma A Avatolicum (as Hyalomma excavatum) from a camel. The infected Hyalomma anatolicum was taken from a pack camal at Sokoto; the infected A variegatum was from the upper Ogun ranch in western state. Others were from animals at the Dugoe abattoir at Ibadan; origins unknown.

Clinical Presentation and Management

Following an infective tick bite, the incubation period is of the order of 1-12 days. The illness begins abruptly with fever, chills, malaise, irritability, headache and severe pains in the limbs and loins followed by anorexia, nausea, vomiting, and abdominal pain. Fever is continuous but may be remittent and sometimes biphasic, resolving by crisis or lysis after 6 days. The face and neck are flushed and oedematous,

the conjunctivae and pharynx are injected, and there is oedema of the soft palate. The mouth is dry and the breath has a foul odour. Patients are depressed and somnolent. In most cases a fine petechial rash begins on the trunk and then covers the entire body. The liver is enlarged in about 50% of cases but the respiratory system is unaffected. A hemorrhagic enanthema appears on the soft palate and uvula early in the illness and other bleeding manifestations, including hematemesis and melena, appear on about the fourth or fifth day in over 75% of patients. Leukopenia and severe thrombocytopenia are common. Large purpuric areas caused by subcutaneous extra vasation of blood occur at times. Bleeding occurs in descending order of frequency from the nose, gums, buccal mucosa, stomach, uterus, intestines, and lungs. Gastric and nasal hemorrhages often lead to death. Involvement of the central nervous system is seen in 10-25% of cases and usually indicates a poor prognosis. It includes neck rigidity, excitation and coma. The mortality rate is often as high as 30-50%, usually due to shock, secondary blood loss, or intercurrent infection.

This severe disease is in sharp contrast to the pattern of disease in Africa, where hemorrhagic phenomena and deaths are only rarely reported.

CHAPTER 3

THE METHOD USED

Research Design and Method

The research design is an ecologic study, also called an aggregate or descriptive study. The unit of analysis is a group. In this study the group is defined as the population in the River Benue Basin. The analysis involves the prevalence of antibody in sera. It was a retrospective study, using sera previously collected in 1965/66.

The primary analytic feature of an ecologic study design is that we do not know the joint distribution of the study factor(s) and the disease within each unit of analysis.

Indirect immunofluorescent antibody technique was used to identify positive sera. The sera were picked at random from a collection of sera that was collected on simple random stratification basis.

Stratification

As laid down by the W.H.O. team that worked on the initial study of Yaws and Malaria in 1965/66.

It was desirable that the sample should be spread out as much as possible over the area that was investigated. Administrative divisions are taken as units of stratification. It was from the 48 survey areas in the River Benue Basin. The River Benue Basin is limited to the west by the River Niger and to the south by the border between the eastern and northern regions and includes roughly the divisions located on both sides of the Benue River.

Selection of Villages

Villages, or the smallest administrative units, were selected in each division according to sampling with probabilities proportional to their population size. The frame was the population figures as published in the population census of the northern region of Nigeria, 1952, Bulletin Nos. 2,4,5,6,10 and 13. The names of the villages or administrative areas selected are given in Figure 1. The geographical distribution of sample points is shown on a map (Figure 4).

Selection of the Population Cluster in a Sample Area

The population cluster of at least 150 persons was surveyed. Donors were selected from the sample area on a household (or house) basis.

Selection of Individuals for Clinical and Serological Examination

All inhabitants of the selected house cluster were examined clinically. Blood obtained from them for serology. Different techniques were used (venipuncture, capillary, etc.). For reasons of convenience, we shall distinguish three types of serological sample, namely, Rondelle Sample, Serum Sample I and Serum Sample II.

Rondelle Sample. Capillary blood by finger or ear-pricking were collected from all children aged 1-14 years and 20% of adults (15 years and over).

Serum Sample I. Blood was collected by venipuncture from one-third of children aged 1-14 years and from the same above mentioned 20% of adults.

Serum Sample II. The venipuncture technique was also applied to children 1-14 years and adults who presented with at least one of the clinical signs of yaws. [Survey design and protocol for the World Health Organization, tre-Panematosis Epidemiological Team (TET) in Northern Nigeria, 1965-1966]. These sera were previously collected by the WHO team doing studies on yaws, virology and malaria, and stored at the Yale Arbovirus Research Unit at -20°C.

The Technique

Viral antibody was detected by the indirect immunofluorescent antibody technique.

Phosphate Buffer Solution (PBS) was the diluent used for the technique. It was prepared by obtaining 20 liters of water, into large containers. One packet of phosphate buffer (Gibeco, Michigan) was added and mixed with a magnetic mixer. This procedure was repeated with a second packet of phosphate buffer, and into the jar of water was added two packets of calcium chloride, mixed as above.

The Staining Technique for the Indirect Fluorescent Antibody Method

The 1:4 dilutions of the sera were made by mixing 0.1 ml sera and 0.3 ml of PBS (diluent) into tubes labelled according to specimen identification numbers, and mixed on a Vortex machine.

On slides with prepared antigen ie.pentavalent antigen, a drop of the diluted sera were added on to corresponding antigen. Each prepared slide has twelve holes. This mixture was incubated on the slides in the humdi chamber for 30 minutes at 37°C, to allow the virus antigen-antibody

reaction to take place. The slides were in plastic boxes that had cotton roll soaked in water to keep up the humidity.

At the end of the incubation period, the slides were washed with PBS and bathed in PBS for 10 minutes. This step was repeated three times, giving a total rinsing of thirty minutes. This removes all traces of antiserum except for those antibodies which are bound to homologous or related antigen. The slides were drained and allowed to dry in air (faster process use blow air). A fluorescein-isothiocyanate (FITC)conjugated anti-globulin was used to identify antibody on the slides. The procedure was as follows:

a. Into a test-tube, 1.8 ml of PBS was added and 0.2 ml of Evans Blue dye was added (1:10).

b. Into a second tube, 2.8 ml PBS and 0.2 ml FIT (MAN) was added.

c. Next, 0.3 ml from tube (a), was mixed with the contents of tube (b).

A drop of the prepared fluorescent media was added onto the slides, and incubated at 27°C for 30 minutes in the humid chamber. This was to allow antigen-antiglobulin reaction to take place.

A drop of conjugated antiglobulin (second antibody) against the human globulin was spread over each preparation. Antibody from virus antiserum, bound to virus antigen in the specimen, now acts as antigen homologous for the antiglobulin conjugate.

The conjugate was rinsed off the slides and the slides were immersed in three changes of PBS for a total of 30 minutes. This removes all

conjugate from the preparations except those fluorescent antibodies which are bound to the virus antigen-antibody complex.

The slides were immersed in distilled water for 2 minutes to remove the PBS. The slides are dried in air and read for fluorescence under the Halogen light microscope using a FITC filter in a dark room. Low power 40X and high power (100X under immersion oil) objectives were used for viewing.

Results of Indirect Fluorescent Antibody Test

Positive immunofluorescence shows greenish-yellow tint on the cells. Positivity is graded from $+^1$ - $+^4$. Negatives show no greenish-yellow tint. The cell simply appears red on a dark background.

Congo Slide Preparation

CER transfer 138 cells were prepared in 150 ml plastic flasks. When they became confluent, we inoculated with CCEF (10200 strain) virus. Cells were observed for cytopathic effect, which was seen on the fourth day (+1 or +2).

To monitor for infection by FA, cells were scraped and a drop of the suspension was applied on to a slide and flamed, then immersed in acetone for 10 minutes at room temperature. A 1:20 dilution of Congo (Dakar 8194) antibody was made. The slides were stained and allowed to stand for 10 minutes. They were washed twice in PBS and soaked for 10 minutes in PBS.

Antimouse (1:40) globulin was added onto the slides, allowed to stand for 10 minutes. They were washed twice in PBS, and soaked in PBS for

10 minutes. Slides were mounted with buffered glycerol and covered with cover slips. They were then read for fluorescence.

To make the Congo spot slides, the media was decanted from the flasks, some saline A was added and rinsed out. This was repeated twice. Trypsin was added and allowed to run over the cell layers. Most of it was decanted and a little left. The CER cell line was allowed to stand at 37°C for 5 minutes. When the cells came off the bottle a little PBS was added and aspirated to break up the clumped cells. The cells were centrifuged at 1500 R.P.M. for 5 minutes. The process was repeated three times. The cells were aspirated from the bottom of the centrifuge tube and a cell count done. The cells were adjusted to 20,000 cells per 0.025 ml.

At this point, one drop of the cells was carefully placed in each spot with a 1 cc. syringe with 27 gauge needle, (1/4 inch) and allowed to dry at 37°C. The slides were fixed with cold acetone for 5 minutes. The process was repeated with cold acetone.

Reason for Choosing the Method

The immunofluorescent technique was the most rapid specific method of serological diagnosis since neutralization tests have not been discovered for these viruses. Antigen slides (except CCHF) for the Hemorrhagic Fever viruses were prepared and inactivated at the U.S. Centers for Disease Control. They were graciously supplied by Drs. K. Johnson and J. McCormick. Viruses for study were selected because of their pathogenicity for man or animals, their unknown distribution in Africa and the lack of knowledge about their distribution in Nigeria prior to great outbreak of disease

During the course of the last 5 years the IF test has become the choice method for serological diagnosis of current disease caused by arenaviruses and the Marburg - Ebola agents. The main reason for the preeminence acquired by the IF in these diseases is that at the present time no other test is available of similar simplicity, sensitivity and accuracy. The method has also been available to the diagnosis of arbobiruses, although in their case, alternative techniques are available. Early reports on the use of the IF technique are: Colorado Tick Fever (Emmons, et al., 1969)(55); Bolivian Hemorrhagic Fever (Peters, et al., 1973) (56); LCM (Deibel, et al., 1975) (57); Argentinian Hemorrhagic Fever (Grela, et al., 1975) (58); Lassa Fever (Wulff and Lange, 1975) (34); CCHF (Zgurskaya, et al., 1975; Burney, et al., 1980) (59,60); Marburg and Ebola Fevers (Webb, et al., 1978; Brown, et al., 1978) (61); and Korean Hemorrhagic Fever (Lee, et al., 1978; Lee, et al., 1980) (62,63).

Serological surveys arrived at detecting past activity of some of the viruses covered by this proposal has been done by plaque reduction neutralization test (PRNT) with Lassa virus (Henderson, et al., 1972; Arnold and Gary, 1977) (64,65) and by intracerebral mouse neutralization with CCHF (David-West, et al., 1974; Saidi, et al., 1975) (54,66). Neutralization test results with these viruses is not easy due either to difficulty in demonstrating antibody development in confirmed cases (Monath, 1975) (67), or to the presence of non-specific virus inhibitions

(Casals and Tignor, 1974) (53) or to the complicated procedure that is used in the test (Botchin and Kinch, 1975) (68). There seem to be no reports in the literature of surveys in the general population with the neutralization test with Junin, Machupo, Marburg and Ebola viruses; even if such test were developed and found otherwise satisfactory for these viruses, the need for conducting it in a Class 4 facility would make the test too restricted and cumbersome.

Serological surveys by complement-fixation (CF) test have limited value with these viruses in that antibodies are relatively short-lived or diminish in titer rapidly in Lassa Fever (Wulff and Lange, 1975; Casals, et al., 1975) (34,69). Persistence of CF antibodies for 3 to 5 years with diminishing titers has been reported in Crimean Hemorrhagic Fever (Karinskaya, et al., 1974) (70). No reports are available with Ebola and Marburg viruses.

The above described difficulties and limitations in the neutralization and CF tests make them less than adequate for a serosurvey; by contrast, the IF technique is simple and reproducible. The final choice should be made on the basis of comparative sensitivity, specificity and persistence of antibodies; such comparison between IF and neutralization test is on the whole, not available. Concerning IF and CF tests, antibodies against Lassa Fever virus are longer-lasting by IF than by CF (Wulff and Lange, 1975; Casals, 1978) (34, 69). The IF test has been successful to practical use in surveys for antibodies against Lassa

Fever virus in Liberia (Frame, et al., 1979; Bloch, 1978) (10,71); against Ebola and Marburg in Zaire, Cameroun and Uganda (Johnson, 1980). The only limitation to the use of the IF test is that a source is needed to supply inactivated spot-slides for Class 4 viruses; in connection with this study, the source was USA MRIID and/or CDC.

Hypothesis

Marburg, Lassa, Ebola, RVF and CCHF viruses are active in a large section of Africa mainly in the tropics and even, at least with RVF, beyond. Disease in man caused by these viruses appears sporadically, either in large outbreaks or in small numbers; in a section of the continent, Lassa Fever is known to be endemic. In addition to severe forms, subclinical infection and mild disease occur with Lassa virus, probably also with Ebola virus; information concerning Marburg and CCHF viruses is either lacking or conflicting. Antibodies detectable by IF test persist for from one to five years, or more, with Lassa infection; after CCHF, IF or complement-fixing antibodies have been reported at least after 5 years from onset. The working hypothesis assumes that surveys as contemplated in this project will increase our information concerning the prevalence of human infection in the areas surveyed.

CHAPTER 4

PRESENTATION AND ANALYSIS OF FINDINGS

Table 1.A shows the total number of sera samples tested for polyvalent antibodies. The polyvalent slides had Congo, Rift Valley Fever virus, Ebola Sudan, Ebola Zaire, Lassa and Marburg (CRE_2LM) antigen on one spot. There were six hundred and two (602) positive of the one thousand and eight (1,008) tested. The percent positive was (60%) sixty.

Table 1.B reflects the figures arrived at after (314) three hundred and fourteen sera tested were subtracted for reasons of not having records on them. (The sampling area was not given.)

Table 1.C, further shows values obtained after data were subtracted for lack of information on age, and sex for two hundred and twenty-four sera. The baseline data now reads 470 tested for CRE_2LM, 193 positive and percent positive 41.

Excluding 538 individuals from sample, may have introduced a bias because the percentage positive is significantly reduced.

Table 2, shows the sample population analysed by age and sex and percentage of the total population. There were 46 percent males and 54 percent females.

Table 3, gives a 37 percent male positive for CRE_2LM and 45 percent female positive for CRE_2LM.

Table 4, Chi-Square Test was done using 2 x K contingency table (comparison of several proportions) p>0.05, not statistically significant, indicating that sampling variation is a likely explanation of females being more antibody positive than males for the viral infections. Tables 4.1-4.5, present individual viruses analysed by age and six.

Table 10, summarizes the study. The approximate antibody prevalences in order of highest to lowest are as follows:

Ebola - 19%
Congo - 14%
Lassa - 9.8%
RVFV - 5%
Marburg- 1.4%

Table 7, shows monotypic, polytypic antibody responses. Dual positives, have 15 possible combinations of two using six (6) viruses. However, 5 combinations accounted for 82 percent (51/62) of all dual infections. Twenty possible combinations of three using 6 viruses, 3 such combinations gave 56 percent (9/16) of all of infections with 3 viruses, only one had infection to four viruses.

Vector Factors

The WHO team in 1965/66 collected data, that revealed the following: Nine of 1,008 or 0.8 percent of the study group left the area where they normally reside for up to one month in ten years. This is a negligible percentage. Hence, all subjects were equally exposed to biting insects (mosquitoes, sandflies, tse-tse flies and other), and rodents in all sample areas insect biting was highly prevalent.

There is animal husbandry in all sample sites. The animals include cattle, goats, pigs, sheep, poultry and dogs.

The main occupation is agriculture, others include fishing, cattle raising, hunting and trading.

Physical characteristics of the survey areas mainly are inland areas (52%). Savannah (17)%, Riverain (21%). The rest are a combination (Riverain and Inland, or Riverain and Swampy or inland, Swampy and Savannah).

Conclusions

Discussion of Findings

There is evidence from the results of the study that the exotic viruses, Congo, RVFV, Ebola Sudan/Zaire, Lassa, and Marburg were present in the River Benue Basin of Nigeria in 1965/66 when the primary data was collected. Rift Valley Fever related infection is present in this region contrary to Tomori's study report (1979a).

Serological survey of Lassa virus has shown that 9.8% of the sample was positive in the sera collected in 1965/66, 15 of 76 males positive, 19% and 31 of 112 females positive, 28%. Statistically, there is no difference in male, female susceptibility $p > 0.05$. However, it goes to agree with work done in Sierra Leone that 6% of the population surveyed had complement-fixing antibody against Lassa virus. Also positive sera was obtained in the Cameroons and Benin. These are all territories in the same geographic and climatic, situations, and hence same vector predispositions. Contrary to the experimental infection study of Tomori (1979a), which stated that there has been no report of clinical RVFV disease in Nigeria. He also went on to state that "no information is available regarding RVFV disease in man in Nigeria, neither has there been any reports of clinical infection nor has any survey for RVFV antibodies in man been conducted". Our study showed, using the indirect

flourescent technique, that 11 of 63 males, 17%, and 14 of 95 females 15% were positive of antibodies to RVFV, projecting a 5% sample surveyed population positive. This is best stated as Rift Valley fever related infection was present in the Benue River Basin when the sera were collected 1965/66 (discussion with Dr. R.B. Tesh*) a neutralization test would categorically confirm if it is Rift Valley fever or not.

This study also showed that 14% of sample surveyed was positive for Marburg virus antibody. Two of 66 males, 3%, and 5 of 100 females, 5% were antibody positive. Distribution on the map, showed areas south of the River Benue and to the east of the territory (See Figure 8). It is interesting to note that males 5-9 years were the only age group affected for males, but for females 5-19 years were the age range, mothers and their children will be the obvious conclusion.

The Ebola viruses constitute the highest prevalence in our study in the area. The Ebola Sudan, however, is commoner than the Ebola Zaire strains in this study. Our study revealed 30 of 62 males, 48% and 41 of 69 females, 59%. Again, using comparison of several proportions, $p > 0.05$, not statistically significant, indicating that sampling variation is the likely explanation of females being more than males. Nineteen percent of sample surveyed was positive.

Of the 49 males tested 10 were positive for Congo virus antibody, 20%, and 22 of 67 females were positive, 33%, a projected value of 14%

*Dr. R. B. Tesh, Yale University School of Medicine, Department of Epidemiology and Public Health, New Haven, Connecticut.

of sample surveyed were positive. These results, compared with the study done at the University College Hospital, Ibadan, in 1973-1974 by David-West et al. shows that the prevalence in 1965/66 of Congo virus antibody in the River Benue Basin is more than that seen in the Ibadan environ (14% to 9.6% total positive; 6% to 20% for males; and 13% to 33% for females) Benue to Ibadan.

The author agrees with the conclusion of David-West (1974), that under favorable conditions, C.C.H.F. is a threat to human health in Nigeria.

Interesting to note is the frequency of antibody response, monotypic infection accounted for 1.9% for RVFV, 5.0% for Ebola Zaire, 7.4% for Ebola Sudan, 6.1% for Lassa, 0% for Marburg, 2.5% for Congo Virus and 10.8% had no infection at all. Duo, triple and quadruple infections were seen in different combinations (See Table 7).

Common factors of exposure predominate in the area studied. The main occupation is agriculture, and they are exposed to various animals and live on similar terrain.

Clustering seen on the various sampling sites for Ebola Zaire and Sudan is indicative of close contact mode of transmission as reflected by the initial history in the Zaire of the first epidemic of the viral infection.

RVFV was seen mainly in the eastern part of the study area and south of the river. Lassa and Congo were spread out both to the north and south of the river, everywhere.

The results presented here deserve further discussion in relation to several obvious questions and problems in interpretation. The first question concerns the specificity of the reactions. The reading of each well on every CRE_2LM slide was verified by an independent observer. Positive reactions were only recorded when the two observers agree. When the same series of sera was retested, there was 87% agreement in reading. The uneven tendency to read positive (4%) or negative (9%) on a second reading could lead to some false positives on CRE_2LM slides in this study. However, the agreement in multiple readings by one reader on monovalent slides was remarkably good (>95%). There were some CRE_2LM positives which were not positive on a single monovalent slide. Since the monovalent slide was the source for the ultimate reading, the margin of false positives was probably quite low. In addition, during a concurrent survey of human sera from the Sudan, different percentages of positive reactions were observed. For example, CCHF positive were rarely found in the Sudan whereas they were frequent in Nigeria.

The relatively high proportion of CCHF positives in Nigeria raises a second question since severe disease has not been described. There are several possible explanations. First, the illness may have gone undescribed. As mentioned earlier, malaria may serve as the great umbrella.

Secondly, the disease course in Africa could be mild because the virus circulating there is antigenically and/or biologically different from Eurasian virus. Despite the paucity of serious case histories in

Africa, there are no scientific data to indicate that the virus is less virulent in Africa than in Eurasia or that more serious cases might not occur in the future (Hoogstrall, 1979) (22). Since CCHF virus is a Nairovirus, it could be that the CCHF virus positives reflect cross-reacting antibodies to a related virus. For example, these tests could be detecting Dugbe virus infections. The answer to this question could and should be resolved by adsorbing positive human sera with CCHF, Dugbe, or other heterologous antigens and looking for residual fluorescence.

A similar procedure could be followed with phlebovirus antigens using inactivated RVF antigen and other heterologous viruses including Gabek Forest and Arumowot viruses which have been isolated in Nigeria (45). Lassa virus positives could be confirmed using heterologous antigens even though they are not known to be in Nigeria.

Other techniques could be used in confirming the results observed in this serosurvey with Lassa, Marburg, Ebola, CCHF and RVF viruses. A test widely used with arboviruses, Hemagglutination-Inhibition (HI), has only limited application with these agents; no agglutinating antigens are available for Lassa, Marburg and Ebola viruses; it is possible, but not easy to prepare an inactivated antigen with some strains of CCHF virus. The Congo virus positive reaction could be confirmed by HI or neutralization. The Elisa, enzyme-linked immunosorbent assay, could be used in a Class 4 facility with the other viruses. However, it is likely that the Elisa test may measure the same viral antigen as the IF. A predominance of nucleocapsid or non-structural antigens, unless purified viral antigens are used.

Despite the obvious shortcomings at present, the majority of our data suggests that our reactions are valid. Assuming that this is the case, what do our results tell us about the distribution of these exotic viruses in Nigeria during 1965-1966? First, they suggest a relatively low prevalence of CCHF virus antibody, Lassa virus antibody and Ebola virus antibody widely distributed over the area surveyed. Regions which were Ebola (Sudan) positive tended also to be Lassa antibody positive also. The distribution of Ebola (Zaire) tended to be more focal suggesting that the Sudan and Zaire strains may have a different epidemiologic history. This observation, although preliminary, would fit current theory which holds that the Sudan strain is an epidemic strain whereas the Zaire strain is an endemic strain.

The distribution of CCHF antibody is in accord with David-West (1975) small clinic-based study of febrile cases in which he found antibody positive in young women with fever. Hospital-based data suffer from a bias in sampling which is not present in this study. Combined with the fact that CCHF virus has been isolated from wildlife in Nigeria, the finding of low prevalence of CCHF antibody in humans is a reasonable did totally expected.

An unexpected finding is the markedly focal localization of RVF antibody in the east, south of the River Benue; Tomori (1979a) found antibody in animals in a different region of Nigeria. The unusual zoogeographical characteristic which could account for this localization

is presently unknown. It could include some property related to vector prevalence or density. It could also be related to movement of livestock.

This is not the first study in which antibody to Lassa virus has been found in the absence of disease (Wulff and Lange, 1975) (34). One possibility is that there are multiple strains of Lassa virus circulating in nature, an epidemic and endemic strain with antigenic differences. McCormick (personal communication, 1983) has found antigenic differences among Lassa virus strains. Obviously, a great many other factors could play a role in converting endemic Lassa infection to epidemic situations.

The results of this study point out vividly how little is known regarding the mechanism by which zoonotic infections become epizootic or epidemic in form. Other retrospective studies in different regions of equatorial Africa could shed additional light on the prevalence of these exotic viruses in nature.

Limitations of Findings and Other Limitations of the Study

The study being purely retrospective and using prevalent cases will not be possible to infer incidence of the viral infections. There is no time of follow-up and there are no controls. The study factors are not known for exposure or outcome.

Conclusions Based on the Study

It is evidenced from the above study that the "exotic viruses" of Congo, Lassa, Ebola (Sudan), Ebola (Zaire), RVFV and Marburg were present

in the River Benue Basin. The author considers these results to suggest that, under favorable conditions, the above mentioned viruses are a threat to human health in Nigeria. They should be looked for in febrile illness that are unresponding to antimalarials and antibiotics; for better health care delivery.

Recommendations

The Federal Government of Nigeria should provide facilities for viral studies and maintain existing viral laboratories in Nigeria. Massive rodent and insect control measures should be embarked upon and surveillance kept up.

The quality of housing should be improved. Proper houses built to replace the mud and thatch huts. Grains should be stored away from human habitation. Education of the general masses will improve understanding of health hazards.

Figure 1

"The Masterlist"

Serum List of WHO Nigeria Treponematosis Survey - in Storage at YARU

Arranged in order of Collection Site Numbers

Collection Site #	Collection Site	Date	Number of Specimens	Specimen Number
01	Begana	15/12/65	26	457-533
02	Eleke	6/12/65	28	272-374
03	Idah Town	18/11/65	27	181-263
04	Emekutu/Ikangpo	17/1 /66	21	647-690
05	Odugbo	13/11/65	33	100-172
06	Oguma (Akuba)	8/11/65	27	1-87
07	Ojobe/Odeke	11/1 /66	28	543-615
08	Ajitata	10/12/65	29	380-444
09	Ekele/Ejule	15/3 /66	17	1495-1537
10	Akwunu/Agadagba	4/2 /66	20	819-905
11	Eloga	19/3 /66	39	1550-1636
12	Emugba	9/2 /66	31	907-990
13	Camp (Ankpa)	28/1 /66	30	708-810
14	Egedde	21/2 /66	24	1092-1161
15	Egedde	14/2 /66	22	1003-1087
16	Utange	23/5 /66	29	2332-2380
17	Mbayem	29/3 /66	20	1643-1702
18	Katisna Alla	31/5 /66	28	2388-2464
19	Mbatser	18/4 /66	30	1796-1873
20	Mbause	18/5 /66	20	2252-2320
21	Nyiev	27/4 /66	30	2968-2060
22	Mbatiav/Mbataska	28/6 /66	27	2768-2830
23	Mbakeritiev	9/5 /66	49	2073-2154
24	Mbapaka	13/5 /66	43	2168-2246
25	Mbayion/Mbaikinde	13/6 /66	31	2616-2687
26	Mbayandella	11/7 /66	13	2834-2847
27	Seneu/Mbaagan	22/4 /66	40	1885-1961
28	Mbaton	21/6 /66	50	2696-2761
29	Ugambe	13/4 /66	33	1715-1785
30	Mbarev-Tiv	19/10/66	25	4519-4582
31	Kumabe	21/10/66	24	4595-4668
32	Ibi Town	28/10/66	38	4673-4745
33	Abong	15/10/66	8	4460-4514
34	Koton Karfi	18/8 /66	39	3525-3596
35	Gulu-Anguwa	9/9 /66	25	3826-3882
36	Dogon Kurhi	28/6 /66	19	3670-3734
37	Loko	9/8 /66	34	3369-3449
38	Shafan Abakwa	13/8 /66	36	3452-3517
39	Keffi Town	22/8 /66	34	3598-3665
40	Gitafa Hausawa	30/8 /66	36	3749-3816
41	Ungan-Madaki	16/9 /66	37	3968-4013
42	Kapanchan Arna	23/9 /66	30	4145-4223
43	Kagaro	29/6 /66	40	4228-4316
44	Agunji	13/9 /66	35	3890-3966
45	Bekyona Biaa	19/9 /66	40	4052-4135
46	Kwandare/Randawesi	21/7 /66	31	3011-3069
47	Madaychiwand	15/7 /66	29	2923-2991
48	Odudu	26/7 /66	82	3087-3192
			1487	

Figure 2

Map of Nigeria, Showing Jos, the Environ of Lassa Fever's First Reported 1969

Figure 3

Map of Africa, Showing Nigeria on the West Coast of Africa

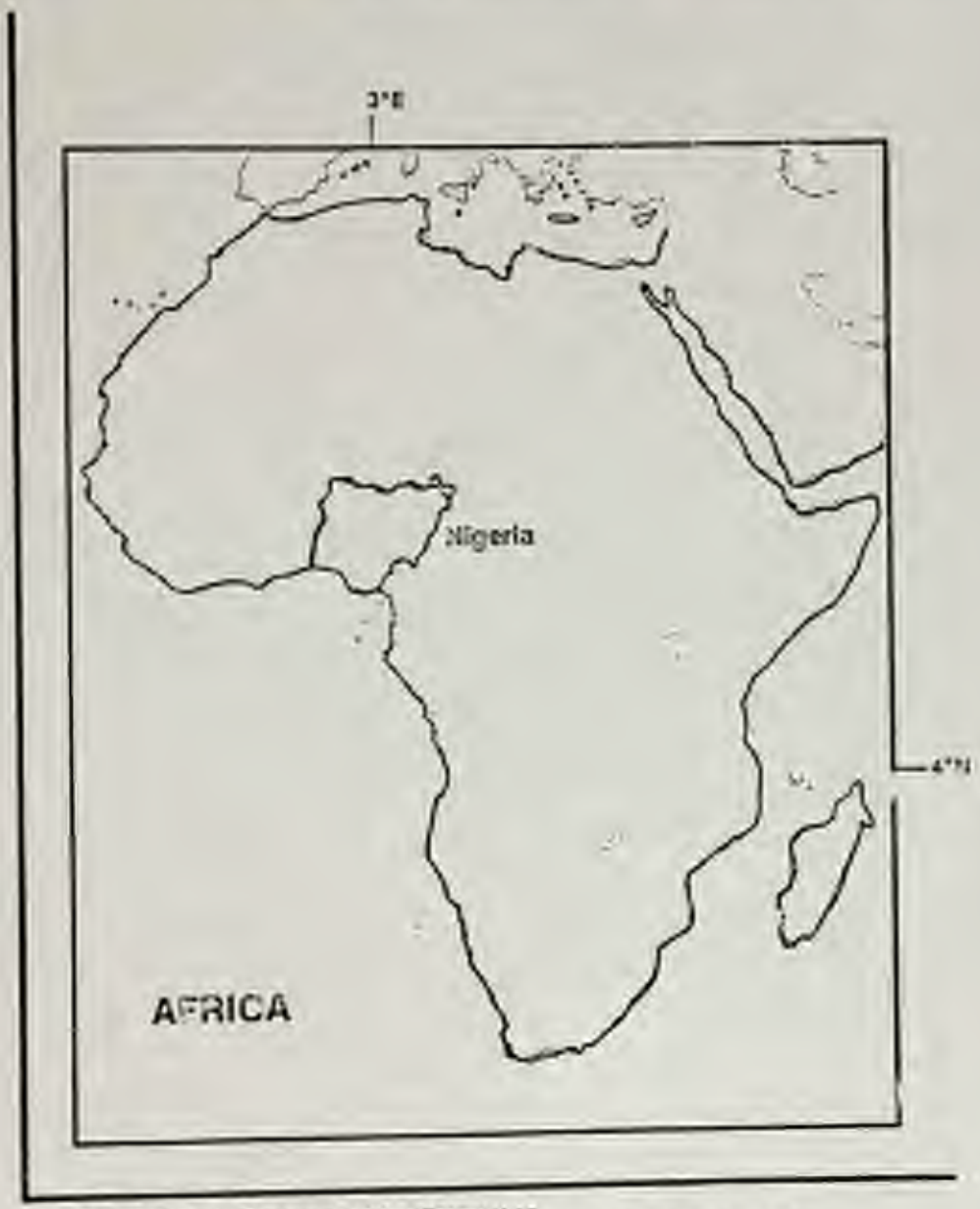
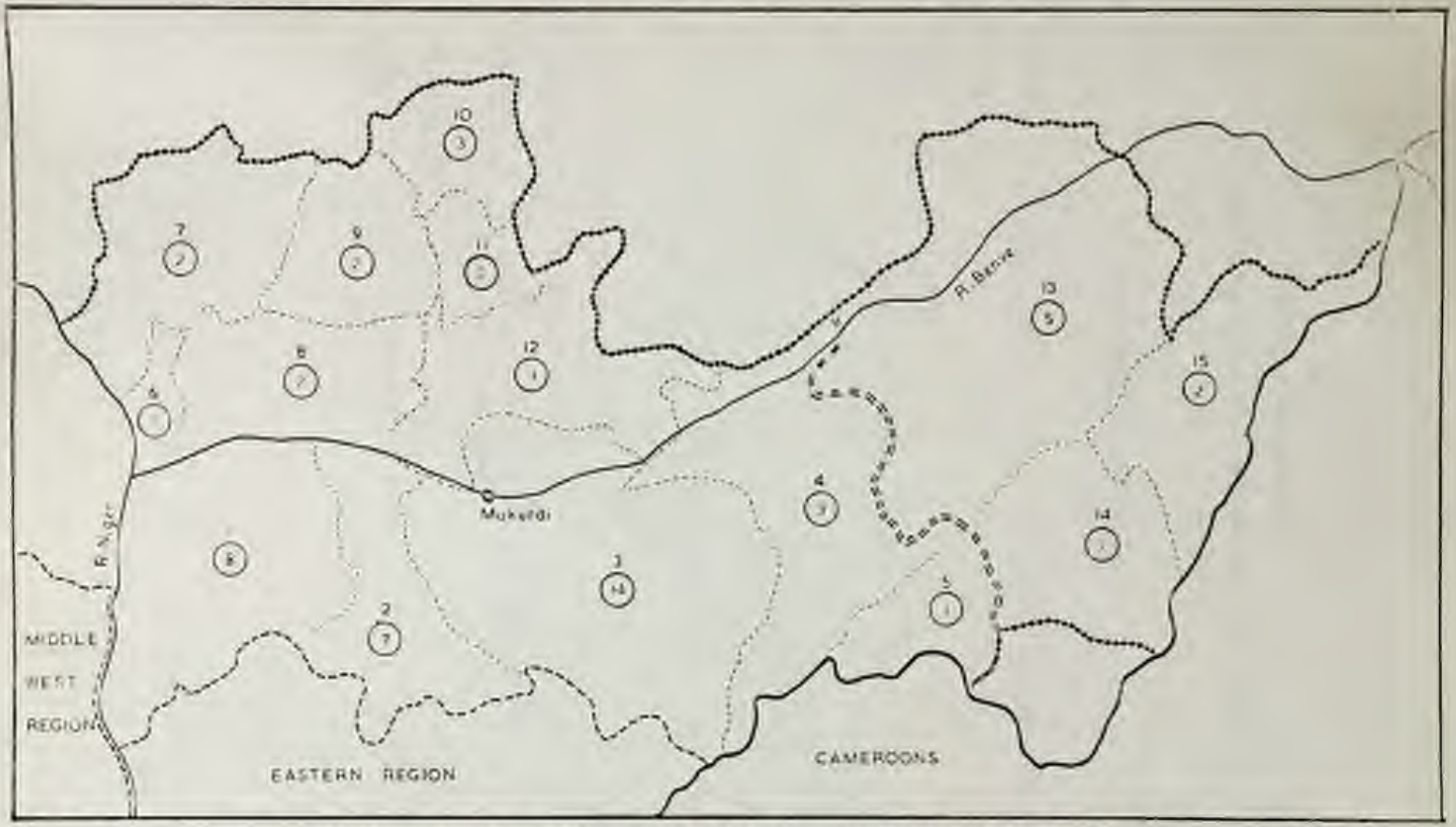


Figure 4. Northern Nigeria - Number of Sample Points in Each Division[1]



[1]The number of sample points is shown in a circle. The division No. corresponds to the stratum No. given in Table 1

Figure 5

Antibodies Incorporated

(Shows the Composition of the Fit (Man) the Human Conjugate)

IMMUNOCHEMICAL CREDENTIAL

PRODUCT: Fluorescent anti-human polyvalent immunoglobulins

PRODUCED IN: Goat

LOT NO: IFL84Y

TITER: 4.68 mg antibody/ml, minimum

ANALYSIS: IEP vs. NHS

Total protein =
16.6 mg/ml
F:P = 2.93 ug/mg

IFL84Y, undiluted

IFL84Y, 1:2

STERILE FILTERED: November 6, 1981

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Figure 6 Distribution of Lassa Virus and Congo Virus in River Benue Basin

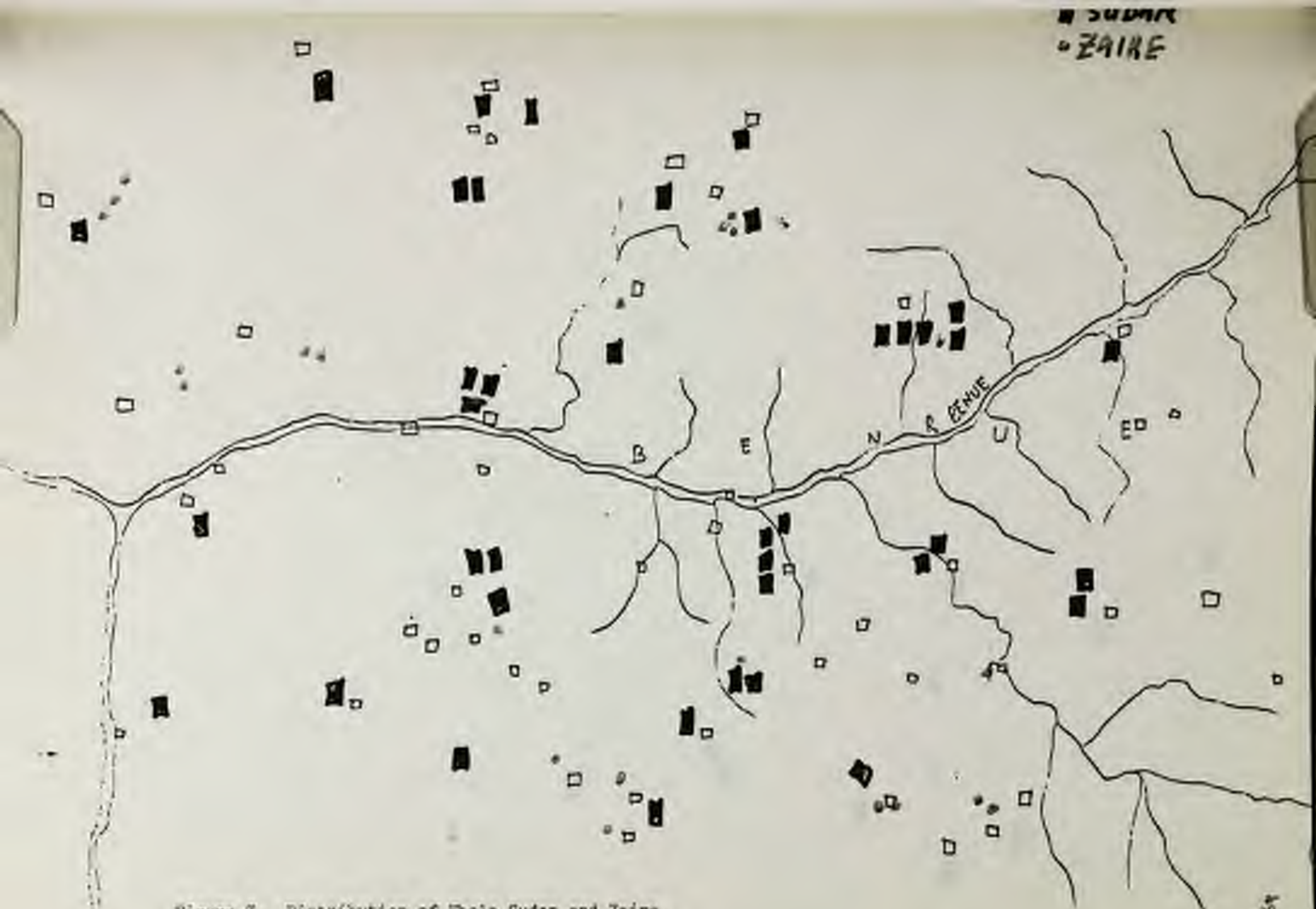

Figure 7 Distribution of Ebola Sudan and Zaire

Figure 8 Distribution of serum% Virus and Rift Valley Fever Virus

Table 1. Haemorrhagic Viruses in Nigeria (Benue River Basin)

An Ecologic Study (Retrospective) (1965/66)

	Tested on CRE_2LN	# Positive	% Positive
1A	1008	602	60
1B	694	438	63
1C	470	193	41

1A. Total number of sera samples tested.

1B. Total number of sera samples tested, having discarded 314 samples, for not having sampling areas on the Masterlist.

1C. Finally, total number of sera samples used for analysis.

Table 2. Population Tested from Benue River Basin Nigeria 1965/66

Sex	A G E - IN YEARS								
	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	42	57	42	29	23	7	9	8	217
Female	41	55	51	50	31	11	9	5	253
Percent of Total Population	18	24	20	13	11	4	4	3	

Table 3. Age and Sex Distribution of CRE_2LM Positives Benue River Basin, 1965/66

Sex	AGE - IN YEARS								
	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	13/42	25/27	10/42	12/29	12/23	3/7	4/9	1/8	80/217
% +	31	44	24	41	52	43	44	13	37
Female	10/42	28/55	18/51	21/50	18/31	7/11	6/9	5/5	113/253
% +	24	51	35	42	58	64	67	100	45

% of population which is CRE_2LM positive = 41%

Table 4. Summary of Virus Antibody Studies in Benue River Basin, 1965/66

	Congo	RVFV	Ebola Zaire	Ebola Sudan	Lassa	Marburg
Total Tested	116/93	158/193	131/193	190/193	166/193	
Males	49	63	62	78	66	
Females	67	95	69	112	100	
% of CBE_2LM Positive	60	81	68	98	86	
Positive by Sex						
Males	10/49	11/63	30/62	15/78	2/66	
%	20	17	48	19	3	
Females	22/67	14/95	41/69	31/112	5/100	
%	33	15	59	28	5	
Projected % of Sample Positive	14	5	19	9.8	1.4	

$p > 0.05$, male/female difference not statistically significant

Table 4.1 CRE_2LM Positives Tested for Congo Virus Antibody by Age and Sex, 1965/66

Sex	A G E - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	9	14	5	8	7	3	3	0	49
% of CRE_2LM Positives	69	56	50	67	58	100	75	0	61 (80)
Female	8	19	12	10	9	1	4	4	67
% of CRE_2LM Positives	80	68	67	48	50	14	67	80	59 (113)

Distribution of Congo Virus Antibody Positives by Age and Sex

Sex	A G E - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	1	4	0	1	3	0	2	0	11
%	11	29	0	13	43	0	67	0	22
Female	3	5	5	2	3	1	1	0	22
%	38	26	42	20	56	100	25	0	33

Table 4.2 CRE_2LM Positives Tested for RVFV Antibody by Age and Sex, 1965/66

Sex	AGE - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	10	16	10	10	10	3	4	0	63
% of CRE_2LM Positives	76	64	100	83	83	100	100	0	79 (80)
Female	9	24	18	15	15	5	4	5	95
% of CRE_2LM Positives	90	86	100	71	83	71	67	100	85 (113)

Distribution of RVFV Antibody Positives by Age and Sex

Sex	AGE - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	2	0	2	3	2	0	2	0	11
%	20	0	20	30	20	0	50	0	17
Female	0	4	3	1	2	1	2	0	14
%	0	17	17	7	13	20	50	20	15

Table 4.3 CRE_2LM Positives Tested for Ebola Virus Antibody (Zaire and Sudan) by Age and Sex, 1965/66

Sex	AGE - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	13	25	10	11	11	2	3	1	62
% of CRE_2LM Positives	63	56	100	92	92	67	75	100	78 (80)
Female	7	17	10	14	10	4	5	2	69
% of CRE_2LM Positives	70	70	56	67	56	58	83	40	61 (113)

Distribution of Ebola Virus Antibody by Age and Sex

Sex	AGE - IN YEARS 0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Zaire	3	4	0	1	3	1	0	0	12
Male	3	1	3	4	4	0	1	2	18
Female									
Sudan									
Male	4	4	3	1	3	0	3	0	18
Female	0	8	4	3	5	2	1	0	23
Z and S									
Male	2	1	1	1	0	0	2	1	8
Female	0	1	1	4	1	0	1	0	8
% Male	15	4	10	9	0	0	67	100	
% Female	0	6	10	29	10	0	20	0	

Table 4.4 CRE$_2$LM Positives Tested for Lassa Virus Antibody by Age and Sex, 1965/66

A G E - IN YEARS

Sex	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	13/42	22/57	12/42	11/29	11/23	3/7	5/9	1/8	78
% of CRE$_2$LM Positives	31	39	29	38	48	43	56	13	
Female	10/41	26/55	18/51	21/50	19/31	7/11	6/9	5/5	112
% of CRE$_2$LM Positives	24	47	35	42	61	64	67	100	

Distribution of Lassa Virus Antibody by Age and Sex

A G E - IN YEARS

Sex	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	2	5	1	2	3	1	1	0	15
%	15	23	8	18	18	33	20	0	15/78 = 19
Female	2	6	5	5	5	3	3	2	31
%	20	23	28	24	26	43	50	40	31/112=28

Table 4.5 CRE,LM Positives Tested for Marburg Virus Antibody by Age and Sex, 1965/66

A G E - IN YEARS

Sex	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	12	20	9	9	8	3	4	1	66
% of CRE,LM Positives	92	80	90	75	67	100	100	100	83
Female	8	26	15	20	18	3	5	4	100
% of CRE,LM Positives	80	93	83	95	100	43	84	80	88

Distribution of Marburg Virus Antibody by Age and Sex

A G E - IN YEARS

Sex	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+	Total
Male	0	2	0	0	0	0	0	0	2
%	0	10	0	0	0	0	0	0	3.0
Female	0	2	2	1	0	0	0	0	5
%	0	7.6	13	5	0	0	0	0	5.0

Table 5. Summary of Ebola Antibody Studies

	#	%
Tested on Zaire and Sudan	113	58% (193)
Negative on Both	55	49%
Positive on Both	14	12%
Zaire Monotypic	7	6%
Sudan Monotypic	37	33%

Table 6. Summary of Approximate Antibody Prevalences

	V I R U S				
	Congo	RVF	Ebola	Lassa	Marburg
Prevalence	14%	5%	19%	9.8%	1.4%

Table 7. Frequency of Antibody Responses

	V I R U S						
	RVF	Zaire	Sudan	Lassa	Marburg	Congo	None
Monotypic	9	24	35	29	0	12	51
Percentage	1.9	5.0	7.4	6.1	0	2.5	10.8
Polytypic	14	44	44	29	7	25	
Percentage	2.9	9.3	9.3	6.1	1.4	5.3	

Dual Positives

15 Possible Combinations of Two Using 6 Viruses
However, 5 combinations accounted for 822 (51/62)
of all dual infections

	Sudan Lassa	Sudan Zaire	Zaire Lassa	Congo Zaire	Congo Lassa
	18	14	5	10	4
Percentage	3.8	2.9	1.66	2.1	0.85

20 Possible Combinations of Three Using 6 Viruses
However, 3 combinations accounted for 567 (9/16)
of all infections with 3 viruses

	Zaire Sudan Lassa	Congo Zaire Lassa	Rift Zaire Sudan	Congo Rift Sudan Marburg
	4	3	2	
Percentage	0.85	0.63	0.42	0.2

APPENDIX

STATISTICAL ANALYSIS

Comparison of Several Proportions.

The Chi-square approach to the comparison of proportions was easily extended to the simultaneous comparison of several proportions. This was the approach used to compare the male-female prevalence significance. These were independent factors, using a 2 x K contingency table, namely, a table of counts of observations arrayed in two columns and K rows the Chi-square with more than 1 degree of freedom, there is no need for a continuity correction.

$$\chi^2(df) = \Sigma\ (O-E)^2\ /E$$

Where

O = Observed count in a category

E = Expected count in that category if the null hypothesis is true

REFERENCES

1. British Medical Journal, 6112:529, 1978.

2. Frame, J.D., Baldwin, J.M., Jr., Gocke, D.J. and Troup, J.M. Lassa Fever, a New Virus Disease of Man From West Africa.

3. Clinical Description and Pathological Findings. American Journal of Tropical Medicine and Hygiene, 23:1131-1139, 1974.

4. Casals, J. Yale Journal of Biology and Medicine, 48:115, 1975.

5. Johnson, K.M. Viral Infections of Humans. 85-92.

6. Carey, D.E., Kemp, G.E., White, H.A., Pinneo, L., Addy, R.F., Fom, A.L.M.D., Stroh, G., Casals, J. and Henderson, B.E. Lassa Fever: Epidemiological Aspects of the 1970 Epidemic, Jos, Nigeria. Trans. R. Soc. Trop. Med. Hyg., 66:402-408, 1972.

7. Frame, J.D., Baldwin, J.M., Jr., Gocke, D.J. and Troup, J.M. Lassa Fever, a New Virus Disease of Man From West Africa. I. Clinical Description and Pathological Findings. Am. J. Trop. Medicine Hyg., 23:1131-1139, 1974.

8. Monath, T.P., Mertens, P.E., Patton, R., Moser, C.R., Baum, J.J., Pinneo, L., Gary, G.W. and Kissling, R.E. A Hospital Epidemic of Lassa Fever in Zorzor, Liberia, March-April, 1972. Am. J. Trop. Med. Hyg., 22:773-779, 1973.

9. Fraser, D.W., Cambell, C.C., Monath, T.P., Goff, P.A. and Gregg, M.B. Lassa Fever in the Eastern Province of Sierra Leone, 1970-72. I. Epidemiologic Studies. Am. J. Trop. Med. Hyg., 23:1131-1139, 1974.

10. Frame, J.D., Casals, J. and Dennis, E.A. Lassa Virus Antibodies in Hospital Personnel in Western Liberia. Trans. R. Soc. Trop. Med. Hyg., 73:219-224, 1979.

11. Frame, J.D., et al. Lassa Fever, A New, A New Virus Disease of Man From West Africa. I. Clinical Description and Pathological Findings. Am. J. Trop. Med. Hyg., 19:670, 1970.

12. Mertens, P.E., et al. Clinical Presentation of Lassa Fever Cases During the Hospital Epidemic at Zorzor, Liberia. Am. J. Trop. Med. Hyg., 22:780, 1973.

13. Monath, T.P., et al. Lassa Fever in the Eastern Province of Sierra Leone, 1970-72. II. Clinical Observations and Virology Studies on Selected Hospital Cases. Am. J. Trop. Med. Hyg., 23: 1140, 1974.

14. Weiss, K.E. Bulletin of Epizootic Diseases of Africa, 5:431-458, 1957.

15. Heyman, D.L., et al. Ebola Haemorrhagic Fever: Tandala, Zaire, 1977-78.

16. Meegan, J.M. and Shope, R.E. Emerging Concepts on Rift Valley Fever.

17. Tomori. Rift Valley Fever Virus, 1979(a).

18. Bowen, E.T., et al. Comparative Study of Strains of Ebola Virus Isolated From Southern Sudan and Northern Zaire in 1976.

19. The Epidemic of Ebola Haemorrhagic Fever in Sudan and Zaire, 1976. P. Bres. Bull. Wrld. Hlth. Org., 56(2):245, 1978.

20. Richman, D.D., et al. Antigenic Analysis of Strains of Ebola Virus: Identification of Two Ebola Virus Serotypes.

21. Johnson, D.K., et al. Marburg, Ebola, Rift Valley Fever Virus Antibodies in East African Primates.

22. Hoogstraal, H. The Epidemiology of Tick-Borne Crimean-Congo Hemorrhagic Fever in Asia and Africa. J. of Med. Entomology, 15 (4):307-417.

23. Causey, D.R., Kemp, G.E., Madbouly, M.H. and David-West, T.S. Congo Virus From Domestic Livestock, African Hedgehog, and Arthropods in Nigeria. Am. J. Trop. Med. Hyg., 19:846-850, 1970.

24. Causey, O.R., Kemp, G.E., Williams, R.W. and Madbouly, M.H. West African Tick-Borne Viruses. Abstr. Rev. 8, Int. Congr. Trop. Med. Malar. p. 669, (Teheran, September, 1968).

25. Moore, D.L., et al. Arthropod-Borne Viral Infections of Man in Nigeria, 1964-1970. Ann. Trop. Med. Parasitol., 69:49-64, 1975.

26. Casals, J. Antigenic Similarity Between the Virus Causing Crimean Hemorrhagic Fever and Congo Virus. Proc. Soc. Exp. Biol. Med., 131: 233-236, 1969.

27. Chumakov, M.P. Crimean Hemorrhagic Fever. (In English, Misc. Publ. Entomol. Soc. Am., 9:123-200, 1974).

28. Williams, R.W., Causey, O.R. and Kemp, G.E. Ixodid Ticks From Domestic Livestock in Ibadan, Nigeria as Carriers of Viral Agents. J. Med. Entomol. 9:443-445, 1972.

29. Causey, O.R., Kemp, G.E., Madbouly, M.H. and David-West, T.S. Congo Virus From Domestic Livestock, African Hedgehog and Arthropods in Nigeria. Am. J. Trop. Med. Hyg., 19:846-850, 1970.

30. Troup, J.M., et al. An Outbreak of Lassa Fever on the Jos Plateau, Nigeria in January-February, 1970. A Preliminary Report. Amer. Jour. Trop. Med. Hyg., 19:693-696. 1970.

31. Simpson, D.I.H. Marburg and Ebola Virus Infections: A Guide For Their Diagnosis, Management and Control. W.H.O., (Offset Publication, No. 36), 1977.

32. Bowen, E.T.W., et al. Viral Hemorrhagic Fever in Southern Sudan and Northern Zaire. Lancet, 1:571-573, 1977.

33. Bechtelsheimer, H., et al. The Neuropathology of an Infectious Disease Transmitted By African Green Monkeys (Cercopithecus Aethops). German Medical Monthly, 14:10-12, 1969.

34. Wulff, H. and Lange, J.V. Indirect Immunofluorescence for the Diagnosis of Lassa Fever Infection. Bulletin of the World Health Organization, 52:429-436, 1975.

35. Emond, R.T.S., et al. A case of Ebola Virus Infection. British Med. Jour., 2:541-544, 1977.

36. Ackermann, R., et al. Deutsche Medizinische Wochenschrift, 97, 1789, 1972.

37. W.H.O. Weekly Epidemiological Record 50:401, 1977; 51:7-8, 1978.

38. Centers For Disease Control. Rift Valley Fever With Retinopathy - Canada. Morb. Mort. Week Rep., 28:607, 1980.

39. Daubney, R., et al. Enzootic Hepatitis or Rift Valley Fever. J. Pathol. Bacteriol., 34:545, 1931.

40. Laughlin, L.W., et al. Epidemic Rift Valley Fever in Egypt: Observations of the Spectrum of the Human Illness. Trans. R. Soc. Trop. Med. Hyg., 73:630, 1979.

41. Colton, T. Statistics in Medicine, 174-181.

42. Jawetz, et al. Microbiology.

43. Ebola Haemorrhagic Fever in Zaire, 1976. Report of an International Commission. Bull. Wrld. Hlth. Org., 56(2):271-293, 1978.

44. Johnson, K.M., et al. Isolation and Partial Characterization of a New Virus Causing Acute Haemorrhagic Fever in Zaire. Lancet, 1: 569-571, 1977.

45. Graham, Kemp, G.E., Causey, O.R., Setzer, H.W. and Moore, D.L. Isolation of Viruses From Wild Mammals in West Africa, 1966-1970. Journal of Wildlife Diseases, Vol. 10, July, 1974.

46. Atkins, J.L., Freeman, S., Schrack, D.W., Jr., Downs, W.G. and Corona, N.C. Lassa Virus Infection. Morbidity Mortality Weekly Rep. 19:123, 1970.

47. Leifer, E., Gocke, D.J. and Bourne, H. Lassa Fever, a New Virus Disease of Man From West Africa. II. Report of a Laboratory-Acquired Infection Treated with Plasma From a Person Recently Recovered From the Disease. Am. J. Trop. Med. Hyg., 19:677-679, 1970.

48. Buckley, S.M. and Casals, J. Lassa Fever, a New Virus Disease of Man From West Africa. II. Isolation and Characterization of the Virus. Am. J. Trop. Med. Hyg., 19:680-691, 1970.

49. McCormick, J.B. and Johnson, K.M. Lassa Fever: Historical Review and Contemporary Investigation,in Ebola Virus Hemorrhagic Fever (S.R. Pattyn, Ed.). 279-285, Elsevier/North-Holland, Amsterdam, 1978.

50. Bishop, D.H.L., Calisher, C.H., Casals, J., Chumakov, M.P., Gaidamovich, S., Hannoun, C., Lvov, D.K., Marshall, I.D., Oker-Blom, N., Pettersson, R.F., Porterfield, J.S., Russell, P.K., Shope, R.E. and Westaway, E.G. Bunyaviridae. Intervirology, 14:125-143, 1980.

51. Rice, R.M., Erlick, B.J., Rosato, R.R., Eddy, G.A. and Mohanty, S.B. Biochemical Characterization of Rift Valley Fever Virus. Virology, 105:256-260, 1980.

52. Swanepoel, R. and Blackburn, N.K. Demonstration of Nuclear Immunofluorescence in Rift Valley Fever Infected Cells. Journal of General Virology, 34:557-561.

53. Casals, J. and Tignor, G.H. Neutralization and Haemagglutination-Inhibition Tests with Crimean Hemorrhagic Fever - Congo Virus. Proc. Soc. Exp. Biol. Med., 145:960-966, 1974.

54. David-West, T.S., Cooke, A.R. and David-West, A.S. Seroepidemiology of Congo Virus (Related to the Virus of Crimean Hemorrhagic Fever) in Nigeria. Bull. Wrld. Hlth. Org., 51:543-546.

55. Emmons, R.W., Dondero, D.V., Devlin, V. and Lennette, E.H. Serologic Diagnosis of Colorado Tick Fever. A Comparison of Complement-Fixation, Immunofluorescence, and Plaque Reduction Methods. Am. J. Trop. Med. Hyg., 18:796-802, 1969.

56. Peters, C.J., Web, P.A. and Johnson, K.M. Measurement of Antibodies to Machupo Virus by Indirect Fluorescent Technique. Proc. Soc. Exp. Biol. Med., 142:526-531, 1973.

57. Deibel, R., Woodall, J.P., Decher, W.J. and Schryver, G.D. Lymphocytic Choriomeningitis Virus in Man. J.A.M.A., 232:501-504, 1975.

58. Grela, M.E., Garcia, C.A., Zannoli, V.H. and Barrera Oro, J.G. Serologia De La Fiebre Hemorragica Argentina. II. Comparacion De La Prueba Indirecta De Anticuerpos Fluorescentes Con La Prueba De Fijacion De Complemento. Acta Bioquimica Clinica Latinoamericana, 9:141-146, 1975.

59. Zgurskaya, G.N., Chumakov, M.P. and Smirnova, S.E. Titration by Indirect Immunofluorescence of Antibody to CHF in Drops of Cell Suspension From Infected Tissue Culture. Voprosi Medical Virology, (English Summary), Moscow, 1975, Institute of Poliomyelitis and Viral Encephalitis, 293, 1975.

60. Burney, M.I., Ghafoor, A., Saleen, M., Webb, P.A. and Casals, J. Nosocomial Outbreak of Viral Hemorrhagic Fever Caused by Crimean Hemorrhagic Fever - Congo Virus in Pakistan, Jan. 1976. Am. J. Trop. Med. Hyg., 29:941-947, 1980.

61. Webb, P.A., Johnson, K.M., Wulff, H. and Lange, J.V. Some Observations on the Properties of Ebola Virus. In "Ebola Virus Hemorrhagic Fever" (S.R. Pattyn, Ed.). 91-94, Elsevier/North Holland, Amsterdam, 1978.

62. Lee, H.W., Lee, P.W. and Johnson, K.M. Isolation of the Etiologic Agent of Korean Hemorrhagic Fever. J. Infect. Disease, 137:298-308, 1978.

63. Lee, P.W., Gibbs, C.J., Jr., Gajdusek, D.C., Hsiang, C.M. and Hsiung, G.D. Identification of Epidemic Hemorrhagic Fever with Renal Syndrome in China with Korean Hemorrhagic Fever. Lancet, May 10, 1980, 1025-1026, 1980.

64. Henderson, B.E., Gray, G.W., Jr., Kissling, R.E., Frame, J.D. and Carey, D.E. Lassa Fever: Virological and Serological Studies. Trans. Roy. Soc. Trop. Med. Hyg., 66:409-416, 1972.

65. Arnold, H.D. and Gary, G.W. A Neutralization Test Survey for Lassa Fever Activity in Lassa, Nigeria. Trans. Roy. Soc. Trop. Med. Hyg., 71:151-154, 1977.

66. Saidi, S., Casals, J. and Fagnih, M.A. Crimean Hemorrhagic Fever - Congo (CHF-C) Virus Antibodies in Man, and in Domestic and Small Mammals, in Iran. Am. J. Trop. Med. Hyg., 24:353-357, 1975.

67. Monath, T.P. Lassa Fever: Review of Epidemiology and Epizootiology. Bull. Wrld. Hlth. Org., 52:577-592, 1975.

68. Hotchin, J. and Kinch, W. Microplaque Reduction: New Assay for Neutralizing Antibody to Lymphocytic Choriomeningitis Virus. J. Infect. Dis., 131:186-188, 1975.

69. Casals, J., Buckley, S.M. and Cedeno, R. Antigenic Properties of the Arenaviruses. Bull. Wrld. Hlth. Org., 52:421-427, 1975.

70. Karinskaya, G.A., Chumakov, M.P., Butenko, A.M., Badalov, M.E. and Rubin, S.G. Certain Data on Serological Investigations of Patients Recovered From CHF in Rostov Oblast. Miscellaneous Publications, Entomological Society of America, 9:142-144.

71. Bloch, A. Serological Survey for Lassa Virus Antibodies in Liberia. Bull. Wrld. Hlth. Org., 56:811-813, 1978.

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